

Message

From: Wayne Miller [Miller.Wayne@azdeq.gov]
Sent: 8/17/2017 4:21:04 PM
To: d'Almeida, Carolyn K. [dAlmeida.Carolyn@epa.gov]
Subject: 2017-8-17 - WAFB -FYI - HACH BART test procedures- ejennings questions - ST012 draft final RDRA WP -

FYI -

From: Steve Willis [mailto:steve@uxopro.com]
Sent: Thursday, August 17, 2017 6:52 AM
To: Wayne Miller <Miller.Wayne@azdeq.gov>
Subject: 2017-8-17 - WAFB -HACH BART test - ejennings questions - ST012 draft final RDRA WP - procedures

A follow-up from Eleanor

From: Jennings, Eleanor [mailto:Eleanor.Jennings@parsons.com]
Sent: Tuesday, August 15, 2017 3:14 PM
To: Steve Willis
Cc: Jennings, Eleanor
Subject: RE: ST012 work plan question

Below in my first email is a description of what you NORMALLY would do for this test. You add your groundwater sample and see if you get SRBs. No problem. I spent a little time on the phone with the technical folks at Hatch, and they gladly answered my questions ("what media is used" – "postgate medium C" – "Cool, that's a normal SRB medium"). So, the test itself is still valid if used correctly. Please note the "if used correctly" phrase.

So first thing: Postgate Medium C is a standard microbial growth media that is used to grow a very specific type of SRB. There are many different types of SRBs, and some do better than others on this medium. However, Hatch uses this particular medium in its test vials. What Amec did was then amend the different vials with different levels of sulfate, and (I'm assuming, hoping, praying) a standard quantity (I hope) of GW (hopefully well mixed between inoculations) from UWBZ34 as a form of SRB inoculum.

Per their results,

2-6 g/l sulfate allowed growth

6 g/l sulfate saw inhibition of growth, but growth still occurred

20-30 g/l sulfate reported "minor" inhibition... not sure what "minor" means exactly as it is not further characterized

300 g/l sulfate fully inhibited SRB growth.

Normal Postgate Medium C (the SRB growth medium used in the test vials) has 2 g/l sulfate. So, the above makes sense. The fact that they report some positive growth does show the presence of SRBs in this MWs water, so we also know that.

I would like to know who actually performed the tests, however, to make sure that somebody other than some field-tech did these analyses, before I give them a pass. AMEC kinda went off the microbial reservation with this, but I'm guessing they needed a fast test to be able to respond to the checklist we sent.

FYI only: I'm Ccing myself on this, in case I need to dig up this answer again (as I'm thinking I may need to). Just easier to find it this way, as opposed to all my "sent" emails.... I also copied the above and pasted it into a new comment bubble on my highlighted version of the report.

Eleanor M. Jennings, M.S., PhD
Principal Scientist - Environmental Microbiology and Biogeochemistry
Eleanor.Jennings@Parsons.com
202.302.9996

"Safety Isn't Expensive. It's Priceless."

From: Steve Willis [<mailto:steve@uxopro.com>]
Sent: Tuesday, August 15, 2017 3:30 PM
To: Jennings, Eleanor <Eleanor.Jennings@parsons.com>
Subject: RE: ST012 work plan question

UWBZ34 analytical results are from preliminary data previously sent. That well had 2300 ppb benzene in July 2016, which appears to be the last sample event for that well. I've attached my mostly-updated spreadsheet that has ST012 analytical results.

From: Jennings, Eleanor [<mailto:Eleanor.Jennings@parsons.com>]
Sent: Tuesday, August 15, 2017 12:15 PM
To: Steve Willis
Subject: RE: ST012 work plan question

The test kit works on the premise that sulfate-reducing bacteria produce sulfide. This produced sulfide reacts with ferrous iron in the liquid media, producing a black iron-sulfide precipitate. It's a dirty way to do some quick "are SRBs here, yes or no" testing. My old major prof actually came up with the process for these, and we used to use small vials with a tiny iron nail/brad dropped in the bottom.

So, is it qualitative – yep.

Is it scientifically valid to use these tests to say if SRB are present or not – yep.

Saying it is scientifically valid to try to estimate SRB population size off of the time it takes to form the black precipitate Ummm, not so fast.

At very best, if somebody is really careful about adding the same amount of inoculum to each vial, incubating them all under very controlled and identical conditions (temperature, for example) then very, very general comments can be made ("this location had a lot more SRB than the other location". However, this is a FIELD test, so how are they keeping all of the environmental controls equal between samples. Temp changes (including temp changes that result from glass vials being left out in the sun, turning into little greenhouses, can radically impact the time to see the formation of iron sulfide precipitates. Per the test documents (see attached), this test can take up to 8 days. I seriously doubt somebody checked them every hour for 8 days straight. Thus, how do they know if the precipitate showed up at 5 PM Wednesday or 7 AM Thursday, if nobody checked during the night? I would never try to put an actual population number to that type of reaction rate, however. Just too many variables, especially as no data was provided as to how samples were handled.

The only time we ever used these types of colorimetric SRB tests was in a most-probable number situation, where dilutions were made to extinction, and then rough population sizes were estimated based on the MPN statistical tables. Yes, I'm old enough to remember back to the days of super expensive molecular biology where you didn't run the molecular tests until you had cheap, dirty data to justify the expense. But not today, given that you can send water samples off for a molecular qPCR analysis and for \$200, and in under a week, you have a very accurate (and scientifically defensible) number of SRB.

This test was designed for folks out on a job site (fracking, for example) and they want to do on-site tests on the produced water to see if SRB are around in high numbers. This test was never intended to try to quantify SRBs,

however. Again, the test was really just designed for dirty field work to say if SRB are or are not present, and to give very, very rough ideas (at best) of qualitative population comparisons.

Oh, and if you're wondering if I have lots of highlights and comment bubbles on my PDF for this section, the answer is "Yes!!".

Where did you find the results of UWBZ34? What section? Section 4.2.2 seems to talk only about figuring out the sodium sulfate dose that the SRB can survive.

Eleanor M. Jennings, M.S., PhD
Principal Scientist - Environmental Microbiology and Biogeochemistry
Eleanor.Jennings@Parsons.com
202.302.9996

"Safety Isn't Expensive. It's Priceless."

From: Steve Willis [<mailto:steve@uxopro.com>]
Sent: Tuesday, August 15, 2017 1:59 PM
To: Jennings, Eleanor <Eleanor.Jennings@parsons.com>
Subject: ST012 work plan question

Are you familiar with the HACH BART test kits they discuss in Section 4.2.2. Do their conclusions appear reasonable? They collected a sample from well UWBZ34 for the test; it's located at the SW corner of the site and last had 2300 ppb benzene.